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Response of direct or priming defense against *Botrytis cinerea* to methyl jasmonate treatment at different concentrations in grape berries

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ABSTRACT

This study was conducted to characterize the forms of disease resistance induced by methyl jasmonate (MeJA) in harvested grape berries and to evaluate the impact of the induced resistance on fruit quality. The results showed that MeJA treatment at concentrations from 10 to 100 μmol/L could effectively induce disease resistance against *Botrytis cinerea* and reduce disease incidence in grape berries. The induced disease resistance was tightly associated with increased H_2O_2 generation, enhanced expression of the defense-related gene *VvNPR1.1* and accumulation of stilbene phytoalexins such as *tran*-resveratrol and its oligomer (trans-)ε-viniferin. The expression of the defense-related gene and synthesis of phytoalexins in 10 μmol/L MeJA-treated grape berries were only significantly enhanced upon inoculating the berries with *B. cinerea*, whereas the 50 or 100 μmol/L of MeJA treatment directly induced these defense responses. Hence, we deduce that the low concentration of MeJA (10 μmol/L) triggered a priming defense mechanism, while higher concentrations of MeJA (50 or 100 μmol/L) directly activated defense responses, thus enhancing disease resistance in grape berries. Moreover, the primed grape berries maintained higher contents of soluble sugars and higher DPPH radical scavenging activity and reducing power compared with those expressing direct defense responses. These results indicate that priming of defense is a cost-effective strategy to protect harvested grape berries from *B. cinerea* infection in terms of minimizing quality loss.

1. Introduction

As a horticultural crop that is produced in worldwide regions, grape fruit has great nutritional and commercial importance in terms of its special functional composition and consumption demand. However, the fruit suffers severe loss during long-distance transport and storage, mainly as a result of gray mold decay caused by *Botrytis cinerea* Pers.: Fr (Romanazzi et al., 2007). The negative impact of fungicides on both the environment and human health has encouraged researchers to explore alternative measures for controlling postharvest diseases in grape fruit. Among recent new strategies, disease resistance induced by environmentally friendly elicitors in horticultural crops has emerged as a promising supplement in approaches to postharvest protection (Schirra et al., 2011).

Induced disease resistance of plants is a state of enhanced defense responses against pathogen attack after treatment with various resistance-inducing agents. The mechanisms of induced resistance appear to involve the direct induction of defenses by the inducing agent and/or the priming of defenses that are expressed following a challenge inoculation of a pathogen (Conrath et al., 2002; Hammerschmidt, 2009). Regardless of when induced defenses are expressed, there is a metabolic cost associated with their expression due to the diversion of resources away from plant growth and development (Walters and Heil, 2007). By comparing the costs and benefits of priming to those of the direct induction of defenses in *Arabidopsis* and barley, it was found that priming causes fewer costs than the direct induction of defense and the benefits of priming-mediated resistance outweigh the costs under conditions of disease pressure, suggesting that priming for enhanced defense is a cost-effective strategy of induced resistance (van Hulten et al., 2006; Walters et al., 2009).

Many natural and synthetic elicitors including salicylic acid (SA), benzo (1, 2, 3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), β-aminobutyric acid (BABA), 2, 6-dichloroisonicotinic acid (INA), saccharin, thiamine and riboflavin have been reported to induce priming defense in plants (Conrath, 2011; Arasimowicz-Jelonek et al, 2012). Interestingly, it was found that treatment of *Arabidopsis* with BABA at a low concentration ranging from 10 to 25 mg/L induced priming defense responses against subsequent pathogen infection and only caused a small reduction in growth, while higher concentration of BABA up to 40 mg/L directly and constitutively activated defense responses and resulted in a significant decrease in growth and seed production (van Hulten et al., 2006). Therefore, it is necessary to clarify the forms of

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induced resistance following treatment with different resistance-inducing chemicals under a wide range of concentrations.

Jasmonic acid (JA) and its methyl ester (MeJA), as naturally occurring plant growth regulators, have been proven to be a plant signal molecule that is involve in plant defense mechanisms (Creeman and Mullet, 1997). Low doses of MeJA are capable of inducing systemic resistance in maize, wheat and *Arabidopsis* so as to protect the plants from various diseases caused by hemibiotrophic and biotrophic pathogens (Ton et al., 2006; Desmond et al., 2006; Chen et al., 2011). In our previous studies, MeJA treatment at low concentrations (1 or 10 μmol/L) has been shown effective in enhancing disease resistance and inhibiting disease incidence on postharvest loquat fruit, peaches and Chinese bayberries (Cao et al., 2008; Jin et al., 2009; Wang et al., 2009). However, the specific defense mechanisms involved in MeJA-induced resistance in harvested fruits are largely unknown. Moreover, no information is available regarding the impact of this MeJA-induced resistance on fruit quality. Therefore, the objectives of this study were first to characterize the forms of disease resistance induced by MeJA treatment at an expanding concentration range in postharvest grape berries, and then to evaluate the impact of the induced resistance on fruit quality and antioxidant parameters.

2. Materials and methods

2.1. Fruit material and the pathogen

Grape berries (*Vitis vinifera* L. × *Vitis labrusca* L. cv. 'Kyoho'), without fungicides being applied before harvest, were hand-harvested randomly at commercial maturity from a local vineyard in Wanzhou District of Chongqing City and transported to the laboratory within 2 h. The freshly harvested berries were carefully selected on the basis of uniform size, color and absence of visual infections and physical injuries. The selected berries were then gently surface-rinsed with sterile distilled water to remove dust and any water soluble residue, and air-dried prior to MeJA treatment and pathogen inoculation.

A *B. cinerea* strain was isolated from decayed grape berries and cultured on potato dextrose agar media (PDA; extract of boiled potatoes, 200 mL; dextrose, 20 g; agar, 20 g in 800 mL of deionized water). The spores of *B. cinerea* were harvested from PDA cultures, which had grown at 25 °C for two weeks. Five milliliters of sterile distilled water containing 0.5% Tween-80 was gently poured into a Petri plate culture, and then the spore suspensions were prepared by removing the spores from the sporulating edges of the cultures with a sterilized bacteriological loop and filtering through four layers of sterile cheesecloth to remove adhering mycelial fragments. Spore suspensions were counted in a hemocytometer and adjusted to 1.0×10^5 spores/mL with sterile distilled water.

2.2. Treatment, inoculation and sampling

Grape berries were randomly divided into two groups, which were redivided into five subgroups of 300 berries each. For the first group, each subgroup of fruit was placed in a 40 L airtight container for MeJA treatment at concentrations of 0 (control), 1, 10, 50 or 100 μmol/L at 20 °C for 6 h according to our previous method (Wang et al., 2009). The containers were subsequently opened and ventilated at 20 °C for 1 h, and then the treated grape berries were surface-sterilized with medical grade (75%) alcohol and drained on filter paper at room temperature, and then were uniformly wounded at two sites in the equatorial zone to a diameter of 1.5 mm and depth of 2 mm with dissecting needles. Afterwards, each wound was inoculated with 15 μL of a 1.0×10^5 spores/mL suspension of *B. cinerea* and allowed to gently air-dry for 1 h (Chen and Zhu, 2011). For the second group, the grape berries in each subgroup were treated with MeJA and wounded as described above. Then aliquots (15 μL) of sterile distilled water were pipetted into each wound site, which served as a control for *B. cinerea* inoculation. After treatments, all the grape berries were incubated at 20 °C and approximately 90% relative humidity for 5 days. Each treatment was replicated three times and the entire experiment was conducted twice. The percentage of infected fruit and lesion diameter were recorded at days 1, 3 and 5 during the incubation. Meanwhile, tissue samples of healthy pulp were taken daily during the incubation and frozen immediately in liquid nitrogen and stored at −80 °C until used for RT-PCR analysis of defense-related gene expression, determination of H_2O_2 generation and contents of phytoalexins and soluble sugars as well as assessment of antioxidant capacities.

2.3. Disease evaluation

The grape berries, with visible gray mold rot zone of more than 1.5 mm wide beyond the wounded area, were considered to be infected (Droby et al., 1999). The numbers of the infected berries and their lesion diameters were recorded. Disease incidence was expressed as percentage of infected berries.

2.4. RNA extraction and reverse transcription PCR (PT-PCR) analyses

Ten grams of frozen sample was ground to a fine powder in liquid nitrogen. Then total RNA extraction was performed using 0.1 g of the powder according to the method described by Liu et al. (2010) with some modifications. According to the manufacturer's instructions, aliquots (1 μg) of RNA were used to synthesize First Strand cDNA using the SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) with an oligo-dT primer, which served as the PCR template. Short and conserved segments of *VvNPR1.1* (GSVIVT00016536001 in Genoscope) were cloned by degenerate primers. Meanwhile, the 18S-rRNA (GenBank ID: AF321266), which is highly conserved and constitutively expressed, was used as a quantitative control for equal cDNA amounts in each RT-PCR analysis. The sequences of primers were designed by the software Primer Premier 5.0 and as follows:

*VvNPR1.1*_forward: 5′-TGTGATAGATTGTTAGCGAGGTGT-3′,
*VvNPR1.1*_reverse: 5′-ATAGTGCGGTCTGCCATCTTTAC-3′,
18S-rRNA_forward: 5′-GCTTTGCCGTTGCTCTGATGAT-3′,
18S-rRNA_reverse: 5′-TTTGCCGATGGTGTAGGTTCCT-3′.

Independent 30 PCR cycles were performed for cDNA amplification linearly related to RNA amounts. RT-PCR products were separated by electrophoresis with 1% agarose gel after staining with ethidium bromide.

2.5. H_2O_2 assay

For H_2O_2 assay, 2 g of frozen sample was homogenized with 10 mL of ice-cold 100% acetone, centrifuged at 10,000 ×*g* for 10 min at 1 °C, after which the supernatant was collected for analysis. The level of endogenous H_2O_2 was determined according to the method of Patterson et al. (1984) by monitoring the absorbance of the titanium-peroxide complex at 410 nm and expressed as nmol per gram of fresh weight (FW).

2.6. Phytoalexin determination

Individual phytoalexins in frozen samples were separated and determined by high-performance liquid chromatography (HPLC) following the method of Verhagen et al. (2010) with some modifications. Phytoalexins were extracted by grinding 5 g of frozen samples in 25 mL of ice-cold 85% (v/v) methanol. After storage overnight at 1 °C, the crude homogenates were centrifuged at 10,000 ×*g* for 10 min (1 °C) and the supernatants were evaporated under nitrogen, dissolved in 2.5 mL of 100% methanol, and then passed though a 0.45 μm membrane filter (Millipore Corp., Bedford, MA) for HPLC analysis of phytoalexins. A 20 μL aliquot of each sample was injected into a reversed-phase Nova-Pak C_{18} column (250 × 4.6 mm, 5 μm), using a linear gradient of 25–80% acetonitrile

within 40 min at a flow rate of 1 mL/min. Individual stilbenes, *trans*-resveratrol and ε-viniferin were measured by an Agilent HPLC series 1100 equipped with 130 Chemstation software and a model G1315B diode array detector coupled to a fluorometer detector (λ_{ex} = 330 nm, λ_{em} = 374 nm) and quantified according to standard calibration curves. Retention times and spectra were compared with those of authentication standards.

2.7. Measurements of soluble sugar contents, DPPH radical scavenging activity and reducing power

Five grams of frozen samples was extracted twice with 50 mL of precooled 95% (v/v) ethanol and then centrifuged at 10,000 ×*g* for 20 min (1 °C) to prepare the extracts. After the ethanol in combined supernatants was removed with a rotary evaporator under vacuum at 30 °C, the residual materials were dissolved in 10 mL of deionized water for measurement of soluble sugar contents, DPPH radical scavenging activity and reducing capacity.

Analysis of individual soluble sugar was performed as described by Cao et al. (2009) with some modifications. Briefly, 5 mL of each extract was passed through a Sep-Pak C_{18} cartridge (Supelco Co., Bellefonte, PA, USA), by which the elute was collected for HPLC measurement of soluble sugar contents. 20 μL of each elute was injected into an a Agilent HPLC equipped with a Zorbax carbohydrate analytical column (150 × 4.6 mm, 5 μm), using deionized water as mobile phase at a flow rate of 1.0 mL/min. Scanning between 190 and 575 nm was performed. Individual sugars such as glucose, fructose and sucrose were quantified using external standardization.

The DPPH radical scavenging activity of the extract was measured according the method of She et al. (2010). The result was calculated according to the following formula: DPPH radical scavenging activity (%) = 100 − (absorbance of sample / absorbance of control) × 100.

The reducing power of the extract was determined according to the method of Bursal and Gülçin (2011). The result was expressed as the absorbance of mixtures measured at 700 nm.

2.8. Statistical analysis

All the experiments were conducted twice using completely randomized design and each treatment was replicated three times. The data presented were from the two independent experiments and expressed as the means ± SE (standard error) of six replicates. All statistical analyses were performed with SPSS 13.0 (SPSS Inc., Chicago, Illinois, US). The data were analyzed by two-way analysis of variance (ANOVA) with treatment and incubation time as factors. The means were separated by Duncan's multiple range test, and differences at $P < 0.05$ were considered to be significant. For percentage values, statistical analysis was carried out after arc sin transformation.

3. Results

3.1. Effects of MeJA treatment at different concentrations on controlling postharvest disease in mock- and B. cinerea-inoculated grape berries

As shown in Fig. 1, the concentration of MeJA applied remarkably affected disease control efficacy. In mock-inoculated grape berries, MeJA treatment at the concentration range from 10 to 100 μmol/L significantly ($P < 0.05$) reduced disease incidence compared with the controls after 5 days of the incubation at 20 °C, whereas low concentration of MeJA (1 μmol/L) had little effect on controlling disease development (Fig. 1A). Similarly, in *B. cinerea*-inoculated grape berries, treatment with 10 to 100 μmol/L of MeJA resulted in significantly ($P < 0.05$) lower disease incidence and lesion diameter compared with the controls during the incubation except for the first day. One μmol/L MeJA treatment had no significant ($P > 0.05$) inhibitory effect on *B. cinerea* infection in grape berries (Fig. 1B and C).

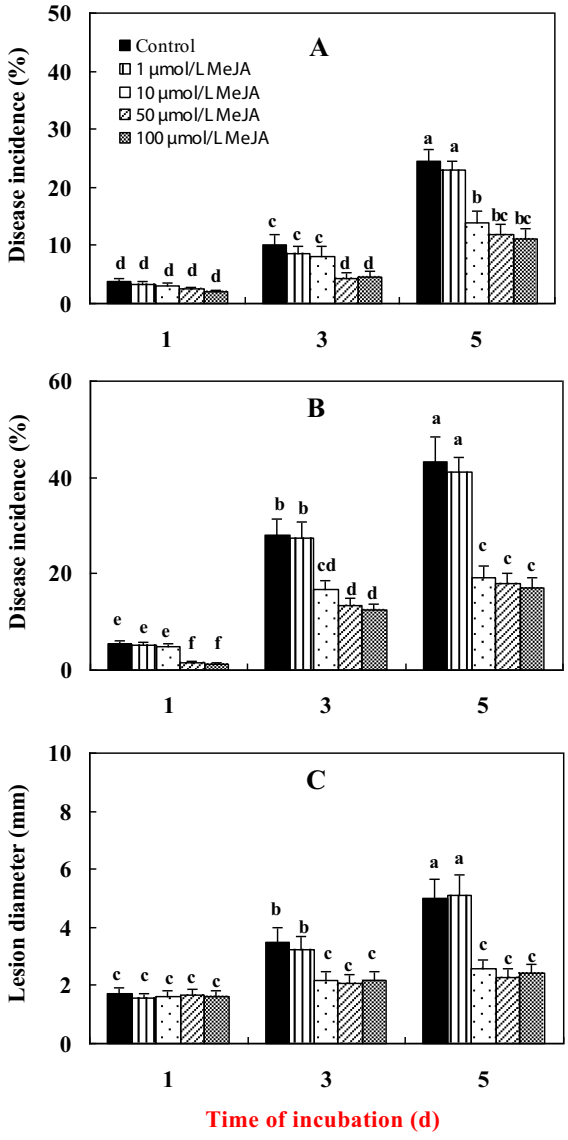

Fig. 1. Changes in disease incidence (A) in grape berries treated with different concentrations of MeJA and inoculated with distilled water as well as disease incidence (B) and lesion diameter (C) in grape berries treated with different concentrations of MeJA and then inoculated with *B. cinerea* during incubation at 20 °C for 5 days. Each column represents the mean of triplicate assays. Vertical bars represent the standard errors of the means. Different letters above the bars indicate statistically significant differences ($P < 0.05$).

3.2. Effects of MeJA treatment at different concentrations on the defense-related gene expression in mock- and B. cinerea-inoculated grape berries

To determine the level of direct and/or priming defense involved in MeJA-induced resistance, we used RT-PCR to quantify the expression of the defense-related gene *VvNPR1.1* in grape berries during inoculation. As shown in Fig. 2, there was no obvious change in the transcript level of the *VvNPR1.1* gene in the berries only inoculated with *B. cinerea* or distilled water. The transcript of the gene was retained at very low level in berries pre-treated with 1 μmol/L MeJA and then inoculated with *B. cinerea* or distilled water. The transcript of the gene was only slightly enhanced in berries pre-treated with 10 μmol/L MeJA and then inoculated with distilled water, however, significantly ($P < 0.05$) higher transcript level was detected upon inoculation with *B. cinerea*, indicating that 10 μmol/L of MeJA treatment induced a priming defense. In berries pre-treated with 50 or 100 μmol/L of MeJA and then inoculated with *B. cinerea* or distilled water, the transcript of the gene

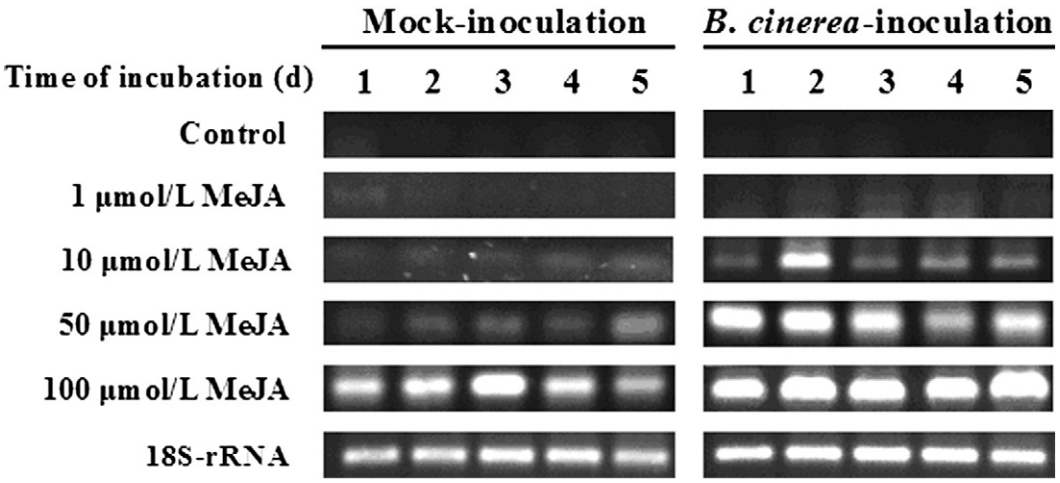

Fig. 2. Expression of the defense related gene *VvNPR1.1* in mock- and *B. cinerea*-inoculated grape berries treated with different concentrations (1–100 μmol/L) of MeJA during the incubation at 20 °C for 5 days. Reverse transcription-polymerase chain reaction (RT-PCR) was conducted using 18S-rRNA as the internal control. All RT-PCRs were made three times from independent experiments with similar results.

was significantly ($P < 0.05$) enhanced and attained a higher level at all the sampling time points compared with the other two MeJA treatments, which suggested that high concentration of MeJA treatment triggered a direct defense.

3.3. Effects of MeJA treatment at different concentrations on H_2O_2 generation in mock- and B. cinerea-inoculated grape berries

Treatment with 1 μmol/L MeJA did not stimulate H_2O_2 generation in mock-inoculated grape berries, whereas the MeJA treatment at 50 or 100 μmol/L significantly ($P < 0.05$) enhanced generation of H_2O_2 during the whole incubation. Meanwhile, the mock-inoculated grape berries treated with 10 μmol/L MeJA exhibited a significant ($P < 0.05$) increase on H_2O_2 generation during the last 2 days of incubation compared with the controls (Fig. 3A). In all *B. cinerea*-inoculated berries, inoculation with the pathogen *B. cinerea* resulted in a rapid and transient burst of H_2O_2, which reached a maximum value at the first day and then declined to background level during the incubation. Pre-treatment with 10, 50 or 100 μmol/L of MeJA resulted in significantly ($P < 0.05$) higher level of H_2O_2 than the 1 μmol/L MeJA treatment and control throughout the incubation (Fig. 3B).

3.4. Effects of MeJA treatment at different concentrations on phytoalexin content in mock- and B. cinerea-inoculated grape berries

In mock-inoculated grape berries, pre-treatment with 50 or 100 μmol/L of MeJA significantly ($P < 0.05$) increased the content of *tran*-resveratrol and ε-viniferin during the whole incubation, whereas no significant change in the phytoalexin content was observed in other two MeJA treatments and the control. However, the ε-viniferin content increased markedly during the first two days and remained at a higher level compared with the control in response to 10 μmol/L of MeJA treatment (Fig. 4A and B). In *B. cinerea*-inoculated berries, pre-treatment with 50 or 100 μmol/L of MeJA significantly ($P < 0.05$) enhanced *tran*-resveratrol and ε-viniferin levels during the whole incubation compared with other two MeJA treatments and the control. Treatment with 1 μmol/L MeJA had no influence on the content of the two phytoalexins compared with the control (Fig. 4C and D).

3.5. Effects of MeJA treatment at different concentrations on soluble sugar content in mock- and B. cinerea-inoculated grape berries

As can be seen in Fig. 5, in mock-inoculated berries, treatment with MeJA at 1 μmol/L did not result in significant reduction in the content

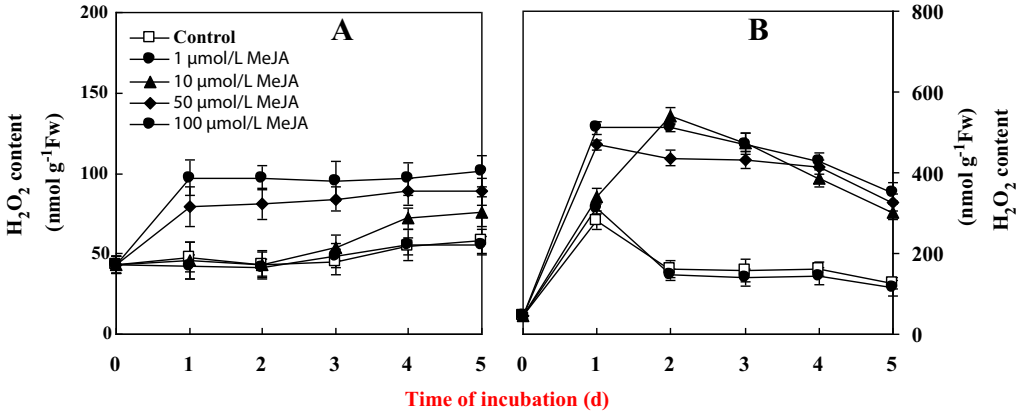

Fig. 3. Effects of MeJA treatment at different concentrations (1–100 μmol/L) on H_2O_2 generation in mock- (A) and *B. cinerea*-inoculated (B) grape berries during 5 days of incubation at 20 °C. Data are expressed as the mean ± SE of triplicate assays. Vertical bars represent the standard errors of the means.

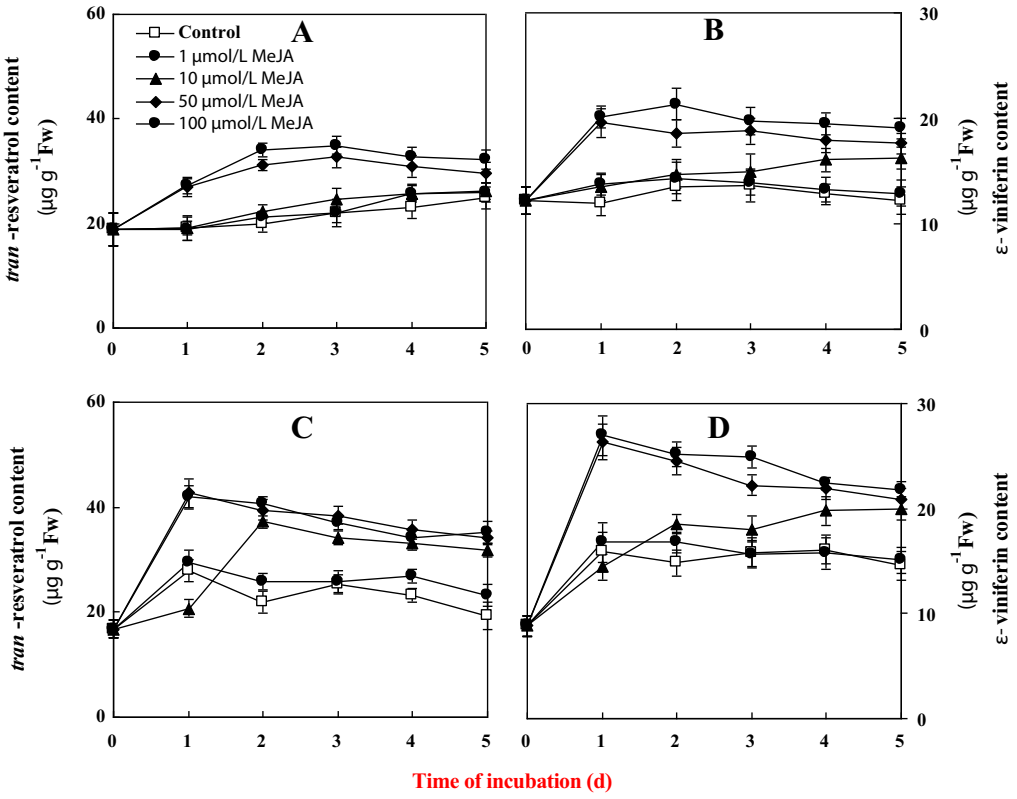

Fig. 4. Effects of MeJA treatment at different concentrations (1–100 μmol/L) on contents of *tran*-resveratrol (A, C) and ε-viniferin (B, D) in mock- (A, B) and *B. cinerea*-inoculated (C, D) grape berries during 5 days of incubation at 20 °C. Data are expressed as the mean ± SE of triplicate assays. Vertical bars represent the standard errors of the means.

of three soluble sugars compared with the control, whereas 10 μmol/L of MeJA treatment maintained significantly ($P < 0.05$) higher soluble sugar contents than the control and 1 μmol/L of MeJA treatment. In contrast, MeJA treatment at 50 or 100 μmol/L reduced glucose content by 17.1% and 19.1%, fructose content by 19.1% and 27.6% and sucrose content by 12.9% or 25.8%, respectively, compared with the control after 5 days of incubation. *B. cinerea* inoculation significantly lowered content of soluble sugars in all berries compared with mock-inoculation controls after 5 days of incubation. MeJA treatment at 1 μmol/L had no significant effect on soluble sugar content whereas 10 μmol/L of MeJA treatment maintained significantly ($P < 0.05$) higher soluble sugar content, in comparison with the control. Pre-treatment with 50 or 100 μmol/L of MeJA resulted in significantly ($P < 0.05$) lower level of the three soluble sugars than the 1 μmol/L of MeJA treatment and the control.

3.6. Effects of MeJA treatment at different concentrations on antioxidant capacities in mock- and B. cinerea-inoculated grape berries

As shown in Fig. 6, in mock-inoculated grape berries, no significant ($P > 0.05$) differences in DPPH radical scavenging activity and reducing power were found between 1 μmol/L MeJA treatment and control after 5 days of the incubation. Treatment with 10 μmol/L MeJA maintained significantly ($P < 0.05$) higher DPPH radical scavenging activity than the control after the incubation. In contrast, MeJA treatment at 50 or 100 μmol/L reduced DPPH radical scavenging activity by 28.1% and 28.8%, and reducing power by 20.2% and 19.5%, respectively, compared with the control after the incubation. *B. cinerea* inoculation significantly lowered antioxidant capacities in all berries compared with mock-inoculation controls after 5 days of incubation. Pre-treatment with 1 or 10 μmol/L of MeJA at had no significant effect on antioxidant capacities in comparison with the control, whereas pre-treatment with 50 or 100 μmol/L of MeJA resulted in significantly ($P < 0.05$) lower antioxidant capacities compared with the other two MeJA treatments and the control.

4. Discussion

Induction of defense responses by various abiotic and biotic elicitors has been inferred to be one of the most widely accepted eco-friendly approaches for controlling postharvest diseases of horticultural crops. The role of jasmonic acid (JA) and its methyl ester (MeJA) as plant defense elicitors in plant-microbe interaction has been extensively investigated. There is increasing evidence indicating that MeJA is involved in defense mechanisms against postharvest diseases through a complex signal network of regulatory interactions in fruit, which stimulated the enhanced expression of pathogenesis-related (*PR*) proteins such as chitinases and glucanases and/or heat shock proteins and accumulation of host-synthesized phytoalexins or other antifungal compounds (Ding et al., 2002; Wang et al., 2014). In this study, we found that treatment with MeJA at concentrations ranging from 10 to 100 μmol/L was effective in protecting grape berries from fungal infection, regardless whether the berries were inoculated with the pathogen *B. cinerea* or not (Fig. 1). Due to the fact that MeJA had no direct inhibitory effect on the spore germination and germ tube elongation of the pathogen *B. cinerea in vitro* (Darras et al., 2005), it is thus proposed that the MeJA treatment inhibited gray mold decay in grape berries by inducing host disease resistance. These results are in good agreement with the capability of MeJA treatment in triggering disease resistance in other fruits in previous reports (Wang et al., 2009; Blanch et al., 2011).

As one of the most peculiar events in the early stages of plant–pathogen interactions, H_2O_2 formation has been reported to exert various effects on plant defense responses, including the reinforcement of the cell wall (cross-linking of structural protein and lignin polymers),

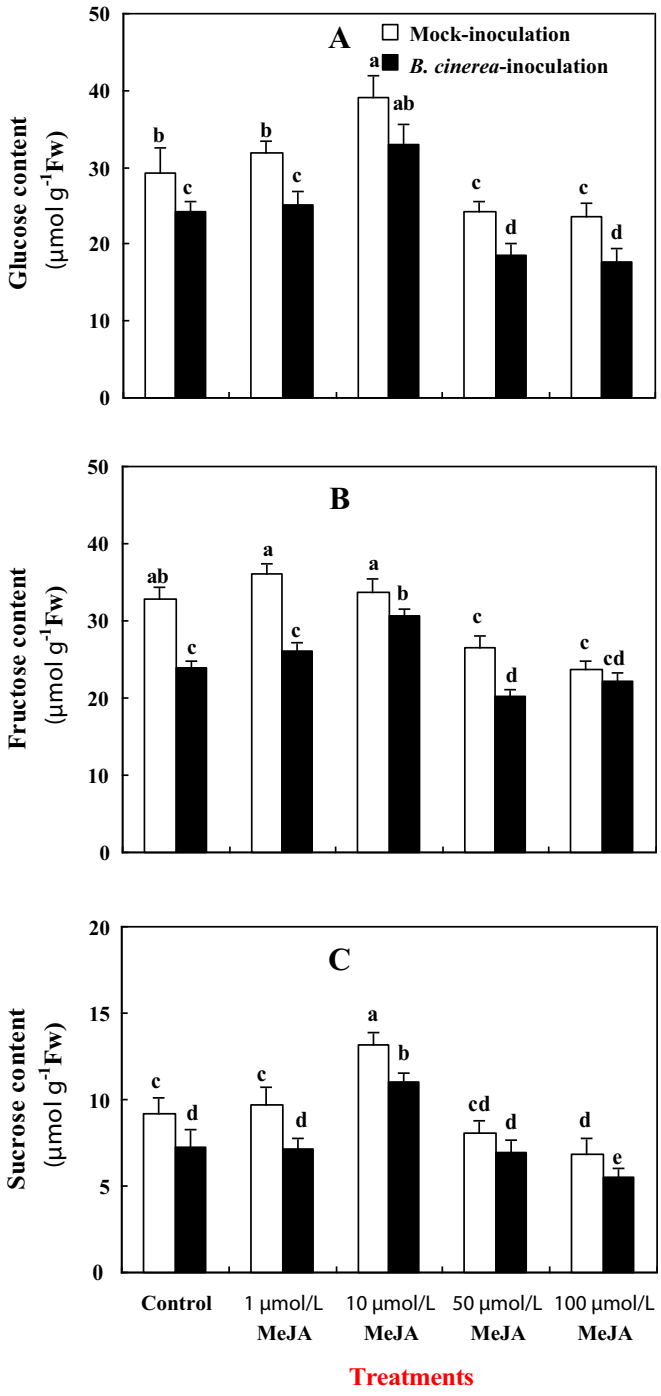

Fig. 5. Effects of MeJA treatment at different concentrations (1–100 μmol/L) on contents of glucose (A), fructose (B) and sucrose (C) in mock- and *B. cinerea*-inoculated grape berries after 5 days of incubation at 20 °C. Data are expressed as the mean ± SE of triplicate assays. Vertical bars represent the standard errors of the means. Different letters above the bars indicate statistically significant differences ($P < 0.05$).

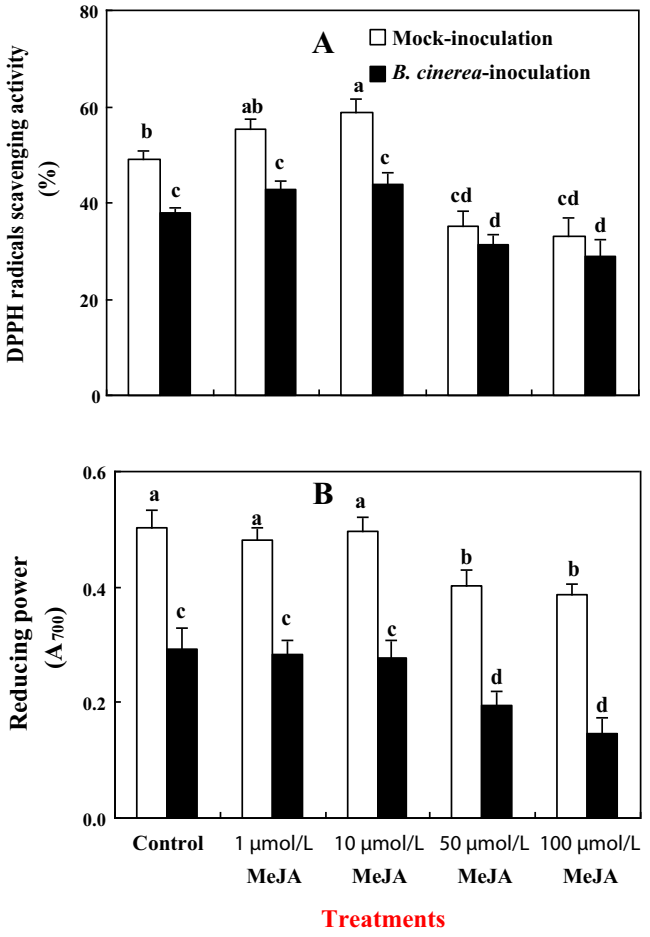

Fig. 6. Effects of MeJA treatment at different concentrations (1–100 μmol/L) on DPPH radical scavenging activity (A) and reducing capacity (B) in mock- and *B. cinerea*-inoculated grape berries after 5 days of incubation at 20 °C. Data are expressed as the mean ± SE of triplicate assays. Vertical bars represent the standard errors of the means. Different letters above the bars indicate statistically significant differences ($P < 0.05$).

hypersensitive cell death, activation of defensive genes, and induction of defensive compounds (Torres, 2010). Timely production of H_2O_2 and resultant prompt activation of H_2O_2-fueled defense responses were highly effective in lowering susceptibility of loquat fruit to *Colletotrichum acutatum*, peach fruit to *Rhizopus stolonifer*, and muskmelon fruit to *Trichothecium roseum* infections, suggesting that the oxidative burst could contribute as a signaling molecule for the induction of disease resistance (Cao et al., 2008; Jin et al., 2009; Ren et al., 2012). Our data showed that MeJA at 1 μmol/L had little effect on H_2O_2 production in mock-inoculated grape berries during incubation, while increasing concentration of MeJA to 50 or 100 μmol/L directly induced a rapid and long-lasting production of H_2O_2 in berries during the incubation, even when the level of pathogenic infection was relatively low. Treatment with MeJA at 10 μmol/L had no effect in inducing the enhancement of H_2O_2 level in mock-inoculated grape berries. However, a strong and transient H_2O_2 burst was observed 2 days after *B. cinerea* inoculation in 10 μmol/L MeJA-pretreated berries, which suggested that 10 μmol/L of MeJA induced disease resistance by priming berry tissue for enhanced accumulation of H_2O_2 upon *B. cinerea* attack (Fig. 3). In line with our results, early rapid production of H_2O_2 was also observed as a crucial facet of the priming responses underpinning JA-induced resistance in rice or *Arabidopsis* against the pathogen *Rhizoctonia solani* or *Pseudomonas syringae*, respectively (Ahn et al., 2007; Taheri and Tarighi, 2010). Similarly, the non-pathogenic rhizobacteria *Pseudomonas* spp. or riboflavin induced priming of defense responses in *Arabidopsis* and grapevine has been proven to involve the H_2O_2 burst in early infection (Verhagen et al., 2010; Boubakri et al., 2013). Thus, we deduced that H_2O_2 may function as a signaling molecule that is crucial for MeJA-induced resistance in grape berries.

VvNPR1.1 gene, which has been identified to play a key role to up-regulate the expression of *PR1* and *PR2* (β-1.3-glucanase) in *V. vinifera* plant, has the potential to enhance defensive resistance upon fungal infection (Le Henanff et al., 2011). The predominant stilbene phytoalexins such as *trans*-resveratrol and ε-viniferin in grapes have been described to possess effective antimicrobial activities against a broad spectrum of pathogens and can be regarded as important characteristics for induced

disease resistance (Boue et al., 2009). In this study, we found that a minimum of 10 μmol/L of MeJA was needed for the induction of disease resistance in grape berries, since a lower concentration of 1 μmol/L was ineffective in inducing the expression of *VvNPR1.1* gene and accumulation of the phytoalexins. These results are in agreement with our previous reports that a threshold of 10 μmol/L of MeJA was essential for the activation of *PR* proteins and the suppression of postharvest decays in loquat fruit and Chinese bayberries (Cao et al., 2008; Wang et al., 2009). Furthermore, the expression of the defensive *VvNPR1.1* gene and accumulation of the two phytoalexins in 10 μmol/L MeJA-treated grape berries were significantly enhanced only upon inoculating the berries with *B. cinerea*, whereas the 50 or 100 μmol/L MeJA treatment directly induced these defense responses (Figs. 2 and 4). The similar characteristics of concentration-dependant defense responses were also reported in BABA or riboflavin induced disease resistance in *Arabidopsis* and tobacco plant (van Hulten et al., 2006; Liu et al., 2010). Therefore, our results suggest that low concentration of MeJA (10 μmol/L) triggered a priming defense mechanism, whereas higher concentrations of MeJA (50 or 100 μmol/L) directly activated defense responses, thus enhancing disease resistance and reducing disease incidence in grape berries. In previous studies, we demonstrated the both *Bacillus cereus* AR156-induced disease resistance against *Rhizopus* rot in peach fruit and the MeJA-induced disease resistance against *Penicillium citrinum* in Chinese bayberries were associated with priming of defense responses (Wang et al., 2013, 2014). These results suggest that priming might be a common phenomenon of induced disease resistance in postharvest fruits.

From an ecological view, the induction of disease resistance can entail costs that allocate limited resources toward production of elevated levels of defense from the plant's own primary metabolism, which will negatively influence plant growth and seed production (Heil et al., 2000). The priming defense was deemed to be of great advantage over direct activation of defense in terms of minimizing the negative effect on crop growth and yield, since the defense responses remained dormant until pathogen infection, thus conferring minor fitness costs under low disease pressure (Van Hulten et al, 2006). The result of Walters and Heil (2007) also indicated that priming-related resistance induced by saccharin significantly inhibited scald pathogen infection in the barley leaf with minor reductions in growth rate and grain yield. The present results demonstrated that the direct activation of defense induced by MeJA at a high concentration (50 or 100 μmol/L) led to a negative impact on fruit quality, as manifested in significantly lower soluble sugar contents and antioxidant capacities in grape berries compared with the non-induced berries, whereas the priming defense using 10 μmol/L MeJA avoided the significant reduction in these quality parameters (Figs. 5 and 6). Therefore, we suggest that induction of priming defense by low concentration of MeJA is superior to direct defense activation by high concentration of MeJA for postharvest disease control in grape berries in terms of minimizing quality loss.

In summary, our data clearly demonstrated that MeJA treatment could effectively induce disease resistance against *B. cinerea* infection and reduce disease incidence in grape berries. Low concentration of MeJA (10 μmol/L) triggered a priming defense mechanism, whereas higher concentrations of MeJA (50 or 100 μmol/L) directly activated defense responses. Moreover, the primed grape berries maintained higher contents of soluble sugars and higher antioxidant capacities compared with those expressing direct defense responses. Therefore, priming of defense is a cost-effective strategy to protect harvested grape berries from *B. cinerea* infection in terms of minimizing quality loss. To our knowledge, this is the first report that disease resistance can be induced through either priming mechanism or direct induction of defense in postharvest fruits, depending on the dose of a chemical elicitor used. Further investigations are needed to elucidate the molecular mechanisms underlying the MeJA-induced priming of defense responses in postharvest fruits.

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